EDO UNIVERSITY IYAMHO
Department of Microbiology
MCB 315 Introductory Virology

Instructor: *Ezeanya Chinyere*, email: ezeanya.chinyere@edouniversity.edu.ng

Lectures: Mondays, 1pm – 3pm, ML1, Faculty of Science building.

Office hours: Mondays, 12noon to 1pm (just before class), Office: Room A6, Faculty building

General overview of the lecture: The course is intended to give the students the basic and important knowledge of viruses. The course entails fundamental topics such as: Introduction to viruses; Historical aspect of virology; General characteristics of viruses; Viral structure and morphology; Cultivation of viruses; Viral replication; Viral detection with cytopathic effects; Classification of viruses; Viral assay and Purification.

Prerequisites: Students should be accustomed with basic classes of microorganism (*e.g.*, types of microorganisms and their diversity) and basic properties of viruses (*e.g.*, non-living nature without a host). Students are also expected to be familiar with commonly known viruses and their infection.

Learning Outcomes: At the end of the course, the student should:

1. Describe how viruses were first discovered and how they are detected.
2. Recognize the basic shapes and morphology of viruses.
3. Understand the classification system for viruses.
4. Discuss the cultivation, assay and replication of viruses.

Assignments: There will be two assignments throughout the course in addition to one test and a final examination. Assignment will be in two parts: Individual and Group assignment. Assignment due date will be communicated and submitted electronically on due date. Home works are organized and structured as trainings and are meant to serve as studying material for the students. There will also be class exercises in groups during the course. The objective of the class exercises is to assess student intellectual ability of the course.

Grading: We will assign 10% of this class grade to assignment (5% for individual and 5% for group), 10% for the mid-semester test, 10% for Class Quiz and 70% for the final exam. The Final exam is comprehensive.

Textbook: The recommended textbooks for this class are as stated:
Title: Basic Medical Microbiology
Authors: Patrick Murray
Publisher: Elsevier, 1st edition
ISBN-9780323478533
Year: 2017

Title: *Medical Microbiology*
Authors: Dr Patrick R. Murray, Dr Ker S. Rosenthal, Dr Michael A. Pfaller
Edition: 7th edition

Title: Microbiology: An Application Based Approach
Author: Michael J. Pelczar, ECS Chan and Noel R. Krieg

Main Lecture: Below is a description of the contents.

Historical Aspect of Virology

Virology is the branch of microbiology that deals with the study of viruses. Dangerous agents were called VIRUSES (Latin virus, poison or venom) in the nineteenth century. Louis Pasteur was the first to describe the virus. He used the term 'VIRUS' to refer to any living disease-causing agent. However in 1892, the first proof of viruses was provided by Dimitri Ivanowski. He discovered that the leaf extract of infected tobacco plant could induce tobacco mosaic disease (TMD) caused by the tobacco mosaic virus (TMV).

There were other scientists that made significant contribution in the early studies of viruses. They include: Felix d'Herelle, who discovered the bacterial viruses (bacteriophages). Prior to that time, Freidrich Loeffler and Paul Frosch (1898-1900) detected that the foot and mouth disease of cattle was caused by a filterable virus.

General characteristics of viruses

Viruses are generally well known as the smallest obligate intracellular parasites ranging in size from 20nm to 300nm in diameter with some recent literature suggesting 30nm to 450nm in diameter. There are basic properties of viruses which cut across different types of viruses. The general features are: Viruses are known to be the smallest infectious microbe when compared to bacteria. At the genetic level, they have only one nucleic acid (DNA or RNA) surrounded by a protein capsid. They lack cellular organization as obligate parasites. They are metabolically inactive and can be crystallized.

Review of RNA Viruses with prevalent infection in Nigeria

1. **Arenavirus:** These viruses are known for their involvement in one of the most prevalent infection currently in Nigeria; the LASSA FEVER (viral hemorrhagic fever). Its structure is sized from 50nm to 300nm. The genome consist of a negative- sense, single-stranded RNA. As an RNA virus, the site for replication in the host is the cytoplasm. As an enveloped virus, release of matured virions (fused within the host cell ribosome) from the host is via budding out of the host plasma membrane.
2. **Retrovirus:** These viruses are known for their involvement in acquired immunodeficiency syndrome (AIDS). Their structure exhibit a typical spherical structure with size of 80nm to 110nm in diameter with its genome containing positive-sense, single-stranded RNA. As an enveloped virus, replicative cycle involves the reverse transcriptase enzyme in the virion which translates the RNA into a DNA in the host plasma membrane with the DNA then incorporated into the host DNA. In maturation and release, the virion releases by budding.
3. **Orthomyxovirus:** These viruses are known for their involvement in influenza infection. Their structure exhibit a typical helical symmetry with size of 80nm to 120nm in diameter with its genome containing negative-sense, single-stranded RNA. As an enveloped virus, replicative cycle involves the budding of the cell membrane during maturation and release. In assembling, this takes place in two different sites in the host: in the nucleus where the nucleocapsid assembles and the cytoplasm where the two surface glycoproteins (neuraminidase and hemagglutinin) assembles.
4. **Paramyxovirus:** These viruses are responsible for causing measles, mumps in humans. It is a larger sized virus (150nm to 300nm) when compared with Orthomyxoviruses. The

genome consist of negative-sense, single stranded RNA. The replicative cycle involves assembling of both the nucleocapsid and its surface glycoprotein (hemagglutinin) in the host cytoplasm unlike for Orthomyxoviruses.

Study of DNA Viruses with prevalent infection in Nigeria

1. Poxvirus: These viruses are known for their pathogenic role in humans causing diseases such as: monkey pox, small pox. They have complex symmetry which are large brick-shaped. The genome contains a double stranded DNA with DNA-dependent RNA polymerase. As a naked virus, replicative cycle takes place inside the cell cytoplasm of the host with release of matured virion via lysis of the cell.
2. Hepadnavirus: These viruses are known to cause hepatitis infection (an inflammatory infection) which destroys the liver of humans. They cause both acute and chronic hepatitis infection. They are generally small sized viruses with 40nm to 45nm. The genome contains double stranded DNA molecules. As a Class 7 virus according to the Baltimore classification system, the replicative cycle involves firstly a repair in the gapped single-stranded DNA, afterwards RNA transcription. Reverse transcription of RNA to produce DNA then follows. However, the reverse transcription takes place within the viral particle.

Reactions to Physical and Chemical agents

Viruses exist defined reactions under certain physical and chemical conditions. Such conditions include:

1. Heat and Cold.
2. Stabilization of viruses by salts.
3. pH.
4. Radiation.
5. Ether Susceptibility.
6. Detergents.
7. Formaldehyde.
8. Photodynamic inactivation.
9. Antibiotics and other antibacterial agents.

Viruses can further be incapacitated by steam under pressure; dry heat; ethylene oxide; sodium hypochlorite, formaldehyde and intra-red irradiation.

Viral structure and morphology

Viruses are known to have simple structure. The structure of the virus entails: Virion (The intact virus unit); Capsid (Protein coat surrounding the nucleic acid core of the virion); Capsomeres (Comprises of a number of subunits); Nucleo-capsid (Arrangement of the nucleic acid and capsid); Envelope (Loose-membrane surrounding the nucleocapsid). Viral morphology is of three basic symmetry: Spherical (Eg; Lentivirus); Helical (Eg; Tobacco Mosaic virus) and Complex (Eg; Poxvirus).

Viral structure exist in certain symmetry. They include: Cubic symmetry of the icosahedral pattern which has 20 faces, 12 vertices with 5-fold, 3-fold and 2-fold of rotational symmetry axes. Secondly, the helical symmetry which are unique for their protein subunits that are bound in a defined pattern to the nucleic acid of the viral particle. Lastly, is the Complex structure, a typical example is the poxviruses which exhibit a complicated brick-shaped structure with ridges on the outer surface.

Cultivation of viruses

Cultivation of viruses is relatively more technical than bacteria or fungi. In virology, cultivation involves the use of whole animal which is still been employed in the studies of viruses. Other modes of cultivation are: cell culture and embroynated egg (fertile egg) under controlled conditions. Cell culture are mainly of three types:

1. Primary cultures which permits a limited number of passages.
2. Secondary cultures resultant of the primary culture supporting up to 50 passages.
3. Continuous cell lines which allows infinite passages.

Cytopathic effects

Detection of growth of viruses on cell culture is observed in the changes of the cell culture morphology. CPE are changes in the morphology of cells: Lysis of the cells; Vacuolation; Formation of syncytia; Presence of inclusion bodies.

Virus classification and Replication

The widely acceptable mode of classification of viruses is the Baltimore Classification system which classifies viruses based on the type of genome and mode of replication. According to this system, viruses are divided into 7 classes: Group 1(double stranded DNA viruses); Group 2 (single stranded DNA viruses); Group 3 (double stranded RNA viruses); Group 4 (positive sense single stranded DNA viruses); Group 5 (negative sense single stranded DNA viruses); Group 6 (single stranded RNA Reverse transcriptase viruses) and Group 7 (double stranded DNA reverse transcriptase viruses).In viral replication, the sequence of viral replication involves: Absorption, Penetration, Uncoating, Biosynthesis and Assembly. The DNA viruses are known to have a replication site in the nucleus whereas the RNA viruses are known to have a replication site in the cytoplasm.

Purification of Viral Particles

Viruses are susceptible to purification resultant of: The exterior surface is made up of proteins; Virus of the same size, shape and density can be segmented in a centrifuge. The methods of viral purification are basically divided into 2 major methods: PHYSICAL METHODS (for example; Filtration, Precipitation, and Centrifugation); CHEMICAL METHODS (Column chromatography, Zone electrophoresis, Use of Specific antisera).The chemical methods is chiefly based on the chemical properties of the virus. Irrespective of the method employed, the aim of viral purification is the achievement of a pure virus particle as without it, certain studies (morphological and molecular) on the understudying virus will not be attained.

Viral assay or Quantification of viruses

Viral assay is a factorial of the infectivity and non-infectivity of the understudy virus. Consequently, the methods of quantification of viruses are based on the **PHYSICAL METHODS** (based on non-infectivity) and **CHEMICAL METHODS** (based on infectivity). In the physical methods, the methods of quantification are: Radio immuno-assays, Enzyme-linked Immuno-absorbent assays. However, the limitations to these methods is the inability of the test to distinguish between infectious and non-infectious viral particles. In the same vein, the use of electron microscopy in the quantification of viral particles can be employed by direct counting against a standard viral particle. Nevertheless, the limitation is similar to the other previously mentioned

physical methods as it is unable to distinguish between infectious and non-infectious viral particles. In chemical methods, the determination of animal death or infection or cytopathic effects at series of dilution is tagged the End-point and expressed as the 50% infectious dose (ID_{50}). The Reed and Munch (1938); the first scientists to determine 50% end-point. Their method for determining the end-point will be explored in this course in comparison with other methods. The plaque assay is also widely employed in the assay of infectious viral particles.

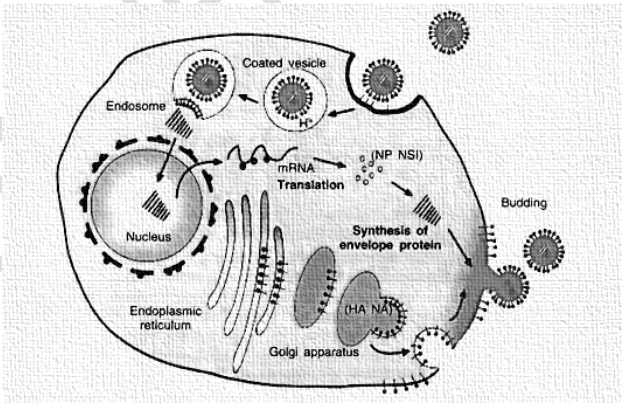
Viral Replication

In viral replication, the sequence of viral replication involves: Absorption, Penetration, Uncoating, Biosynthesis and Assembly. The DNA viruses are known to have a replication site in the nucleus whereas the RNA viruses are known to have a replication site in the cytoplasm. According to the Baltimore classification system, viruses are classes into seven classes: Class 1 to Class 7.

In this lecture, we will examine the replication of a DNA and RNA viruses each.

DNA Viruses (Case study: Adenovirus)

As with all DNA viruses, Adenovirus replicates exclusively in the host nucleus.



ATTACHMENT, PENERATION AND UNCOATING:

The attachment, penetration and uncoating steps are initiated by three viral proteins: pre-terminal protein, DNA polymerase and DNA binding protein.

BIOSYNTHESIS

The DNA of this virus is transcribed asymmetrically to produce mRNA. New mRNA are synthesized prior to DNA replication. The DNA-dependent RNA polymerase transcribes the virus DNA and is connected to the core of the infecting virion.

ASSEMBLY

Assembly of the viral particle originates in the cytoplasm with development of capsomers, although absolute packaging is completed in the host nucleus.

RNA Viruses (Case study: Rhabdovirus)

As with all RNA viruses, the genome consist of RNA but instead have a single-stranded, negative sense RNA. Rhabdovorus replicates exclusively in the host cell membrane.

ATTACHMENT, PENERATION AND UNCOATING:

The attachment, penetration and uncoating is via glycoprotein spikes with the nicotinic acetylcholine receptor serving as receptor for the virus on the host central nervous system.

BIOSYNTHESIS

The RNA is transcribed to produce mRNA by RNA polymerase. Ribonucleoprotein (RNP) acts as the template for transcription. The RNA-dependent RNA polymerase transcribes the virus RNA.

ASSEMBLY

An envelope is acquired by the virus via budding out of the plasma membrane. Spikes are formed on the outer surface of the virus by viral glycoproteins.

Overview of RNA Viruses

RNA viruses replicate via RNA intermediates (requiring RNA-dependent RNA-polymerase). The site of replication in the host cell is the host cytoplasm which is typical to all RNA viruses. Viral proteins are exclusively formed proceeding the production of messenger RNA is available.

RNA viruses exist as either, positive; negative single stranded RNA viruses or double stranded RNA viruses.

Positive- single stranded RNA viruses

Here, the viral RNA is similar as mRNA and acts as mRNA. Consequently, the mRNA can be translated instantaneously on initial infection of the host cell.

Examples:

- Poliovirus
- Togaviruses
- Flaviviruses

Negative-single stranded RNA viruses

The virion RNA is complementary in base sequence to the mRNA. The RNA is complementary to virion RNA and acts as the mRNA. RNA-dependent RNA-polymerase involved in replication is also packaged in the virion so as to serve in producing mRNAs on infection of host cell.

Examples:

- Influenza Virus (Orthomyxovirus)
- Measles Virus, Mumps Virus, (Paramyxoviruses)
- Rabies Virus (Rhabdovirus)

Double-stranded RNA viruses

The virion (genomic) RNA is double stranded and so cannot function as mRNA; thus these viruses also need to package an RNA polymerase to make their mRNA after infection of the host cell.

Example:

- Rotaviruses

Summary/ Conclusions: Virology is a branch of microbiology that deals with the study of viruses. Scientific approach in terms of cultivation, detection, purification, quantification of viruses is well studied and improved with the study of virology.

Interactions and Questions: The questions will be asked during the course to ascertain student understanding of the course. Such questions include:

1. What are the methods of viral cultivation in the laboratory?
2. What is the principle description of viral nucleic acid?
3. What methods can be used to quantitate viral infectious titer?

Bibliography/ Further Readings:

Journal of virology

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